8

Japanese Patent Laid-Open-to-Public

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Specification

1. Title of the Invention

Gradually Emissive Drug

2. Claim

A gradually emissive drug containing a medically active substance suited for hypodermic or muscular administration an hyaluronic acid or a salt thereof.

3. Detailed Description of the Invention

Field of the Industrial Utilization

This invention relates to gradully emissive drugs.

Prior Art

Up to date, for the preparation of drugs extensive researches and investigations have been conducted to let active components of drugs be emitted gradually in the body or on the surface thereof. However, there has been substantially no example of success in any emissive drug

suited for hypodermic or muscular administration.

Summary of the Invetion

As is well known in the art, hyaluronic acid is one of naturally occurring acidic mucopolysaccharides and widely distributed in coupled tissues of animals. Its ecological fitness has already been recognised. It is used as natural moisture retainer for cosmetics, and is utilised as muscular administration substance for improving the function of joint muscles (available under a trademark of "ARTZ" from Kaken Seiyaku) and administration substance (available under a trademark "Opegan" from Santen Seiyaku). Aqueous solution of hyaluronic acid has high viscosity, which is capable of control with the molecular weight, concentration, pH, ion intensity, etc. of hyaluronic acid.

The inventors conducted extensive researches and investigations concerning the control of the emission of medical from the drug by making use of the property of hyaluronic acid. The present invention is predicated in these researches and investigations.

More specifically, the invention concerns a gradually emissive drug, which is suited for hypodermic or muscular administration and coaprises a medically active substance and hyaluronic acid or a salt thereof permissible in drug preparation.

According to the invention, as the hyaluronic acid may be used hyaluronic acid and a salt thereof with a member of the group consisting of alkali or alkali earth metals, aluminum, ammonium and substituted ammonium (hereinafter referred to as hyaluronic acid or a salt thereof). The hyaluronic acid used according to the invention suitably has a molecular weight of about 560,000 to 2,400,000. Hyaluronic acid is highly safe; for instance its D in the hypodermic administration is over the limit of the physical administration (i.e., 1,500 mg/kg (Hidemichi Akasaka et al, "Properties and Applications of Hyaluronic Acid as Biopolymer", Fragrance Journal, No. 78, 1986, p-p 42-47).

According to the invention, any medically active substance may be used so long as it it capable of hypodermic of muscular administration. Its examples are anti-biotic substances, anti-inflammatory substances, anti-bacteria substances, anti-viruses substances, anti-infection substances, anti-cancer substances, cellular propagation suppression substances, wound curing substances, anesthetic substances, medicines for the circulatory system, medicines for the dygestion system, hormones, vitamins, etc. Among these drugs, insulin, crystalline zinc insulin and noncrystalline zinc insulin are particularly suitable from the standpoint of the

conventional method of administration. As is well known in the art, insulin is used for curing the diabetes. It is decomposed by the succus gastricus, and usually it is hypodermically administrated (sometimes several times a day). For this reason, aqueous noncrystalline and crystalline zinc insulin suspensions for injection have been developed. However, they are not satisfactory. In addition, hypodermically administrated insulin has biological utilization power of 50 to 60 % compared to

. This means that the gradual emission is useful.

The hypodermic or muscular administration according to the invention may, for instance, be hypodermic administration by injection or perfusion. It is said that in the hypodermic tissues of animals connective tissues are concentrated in to permit injection of a large amount of liquid medical ("Biologycal Drug Experiment Manual", Shigeru Goto, 1985, issued from Seishi Shoin, page 76). Thus, when injection substance as gradual emission drug is considered, the amount of administration at a time is increased. In addition, when expecting extreme gradual emission, greater amount of gradual emission material is necessary. For this reason, hypodermic administration is convenient.

The drug according to the invention contains the medically active substance noted above and hyaluronic acid or a salt thereof. Suitably, both the components are made to be present in unit administration. For instance, the two are made to be present in ampule or vial such that they are dissolved or suspended in bacteria-reduced water or bacteria-reduced physiological brine. The drug may be prepared by mixing the solution or suspension of the medically active substance and the solution or suspension of hyaluronic acid or a salt thereof or by adding powder of hyaluronic acid or a salt thereof to the solution or suspension of the medically active substance or vice versa. This administration unit may contain conventional additives such as tension uniformalizing agent or local paralyzant. Further, it is of course possible to mix the medically active substance and hyaluronic acid or a salt thereof immediately after the administration, the mixture being used as a solution or a suspension. The weight ratio of the medically active substance and hyaluronic acid or a salt thereof is variable in a wide range depending on the character of the medically active substance. For example, it is 0.01 : 1 : 1 to 100 : 1, preferably 0.01 : 1 to 10 : 1.

Examples

Now examples are given.

Example 1

Contrast insulin for injection was prepared by dilutig swine neutral insulin for injection (Novo Actrabit MC 40 IU/ml) with physiological brine to 0.5 IU/ml.

Meanwhile, the insulin for injection containing hyaluronic acid according to the inventioo was prepared by adding powder of hyaluronic acid (with a mean molecular weight of 1,400,000) to the above diluted insulin for injection such that the concetration of hyaluronic acid is 1 %.

By using these substances for injection, the following animal experiment was conducted.

As the experiment animal were used eight male livestock rabits (Japanese white rabits, each weighing 2.1 to 2.6 kg) in two groups each of four, i.e., a sole insulin group and hyaluornic acid/insulin group. To let insulin be quickly secreted after being taken in the body, fast was done for 24 hours before the administration, thus avoiding variations of the hyperglycemic value. The amount of administration was set to 0.5 IU/kg for each group, and the administration was done hypodermically on the back (22 G, 2.5 ml, with disposable injector manufactured by Terumo). Blood was extracted from auricular vein before the

administration and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after the administration. The hyperglycemic value of the plasma was measured.

The hyperglycelic value was measured by using gucose-B-testkid (GOD-POD, Wakojun Seiyaku).

As for data, hypercglycemic change (%) at the time of measurement was calculated by setting the hypercglycemic value before the administration to be 100 %. Further, overall rate (%) of change in the hypercglycemic value up to 12 hours from the administration was determined by using the following equation.

Rate (%) of change in the hypercglycemic value up to 12 hours from the administration

$$= 100 \times \left(1 - \frac{AUC_0^{19}}{12 \times 100}\right)$$

Table 1 and Figure 1 show changes with time in the hyperglycerimic value change rates obtained with the sole insulin group and the combined hyaluronic acid group.

Table 1

Time (hours)	Sole insulin acid administration mean hyperglycemic value change rate (%)	S.E.	Combined hyaluronic acid administration mean hyperglycemic value change rage (%)	S.E.
0	100.0		100.0	
0.5	95.6	14.3	75.4	6.8
1	81.4	8.5	66.8	6.2
2	49.7	4.8	60.5	2.5
3	45.3	8.2	57.8	1.6
4	44.2	5.0	61.9	5.3
6	47.9	4.2	48.2	3.9
8	80.1	11.2	52.8	3.7
12	98.7	3.4	43.2	6.5*
24	96.9	9.2	103.2	6.8

*: p 0.01 or p 0.05

As is seen from the results of experiment, clear continuity is recognised in the reduction of the hyperglyceric value with the sole insulin group compared to the combined hyaluronic acid group. Particularly, useful difference is present between the two values obtained after 12 hours from the administration (p 0.01 and p 0.05). In the case of the sole insulin, the reduction of the

hyperglycemic value is maximum after 4 hours from the administration and is subsequently restored to the initial vlaue. The rate of change in the hyperglycemic value after 8 and 12 hours from the administration, are 80 and 97 %, respectively. In the combined hyaluronic acid group the hyperglycemic acid change rate is high compared to the values in the sole isulin group after 1, 2, 3 and 4 hours from the administration. After 8 and 12 hours from the administration, it is reduced to be about 53 and about 43 %, respectively. This delay of the restration of the hyperglycemic value in the combined hyaluronic acid group, is attributable to continuous absorption of insulin with combined use of hyaluronic acid.

The hyperglycemic value change rate up to 12 hours after the administration was 32.5 3.4 % with the sole insulin group and 44.6 2.5 % with the conbined hyaluronic acid group, and a useful difference between the two cases was recognized (p 0.05). The fact that this is recognized with the same amount of administration shows that hyaluronic acid is effective for improving the biologycal utility of insulin in the hyperdermic administration.

Similar animal experiment was conducted with hyaluronic acid with mean molecular weights of 500,000,

1,000,000 and 2,000,000. In these cases, continuous absorptive action of insulin with combined use of hYaluronic acid was recognized.

4. Brief Explanation of Drawing

Fig. 1 is a graph showing effects of hyaluronic acid on insulin action to reduce the hyperglycemic value.

Fig. 1 --- Hyperdermic value change rate (%) Time :

Insulin plug hyaluronic acid +: Insulin

Amendment of Procedure (voluntary)

as of May 18, 1989

To the Director-General, Patent Office, Fumio Yoshida

1. Identification of the Case:

Japanese Patent Application No. 116678/88

2. Title of the Invention:

Gradually Emissivre Drug

3. Amending Party:

Relation to the Case: Applicant

Address: (Post No.: 142), 2-17-11, Nakanobe,

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Name: Roman Kogyo Co., Ltd.

Representative: Tameo Hiramori

4. Subject of Amendment:

Detailed description of the invention in the specification

5. Content of Amendment:

(1) In the specification, on page 9, between the bottom but 2 line and the bottom line, insert the following.

Example 2

As glucagon for injection was used what is prepared by

diluting cattle or swine glucagon for injection (glucagon NOBO for injection 1D.S.P./vial) with physiological brine to 0.05 U.S.P./ml. As hyaluronic acid-added glucagon for injection was used what was obtained by diluting the glucagon for injection noted above with physiological brine to 0.05 U.S.P./ml and then adding hyaluronic acid powder (molecular weight: 1,400,000) to a solution containing 10 % of hyaluronic acid.

As the experiment animal, eight normal male rabits (Japanese white rabits weighing 1.9 to 3.2 kg) were used in two groups each of four, i.e., a sole glucagon group and a hyaluoronic acid acid-added glucagon group. A fast for 24 hours was made before the administration. The amount of administration was set to 0.05 U.S.P./kg. The administration was done hypodermically on the back. Blood was extracted from auricular vein before the administration and 0.25, 0.5, 1, 2, 3, 4, 6 and 8 hours after the administration to measure the hyperglycemic value of plasma. The hyperglycemic value was measured by using glucose-B-testkid (GOD-POD, Wakojun Seiyaku).

The result is shown in Table 2.

Table 2

Time (h)	Sole glucagon group hyperglicemic value change (mg/dt)		HA-added (1 %)) glucagon hyperglicemic value change (mg/dt)	
0	0.0		0.0	
0.25	49.1	21.2	54.3	19.2
0.5	50.8	22.3	50.2	13.4
1	62.9	29.7	51.0	15.6
2	30.5	25.2	31.7	8.9
3	25.2	18.9	17.3	14.6
4	17.2	13.6	24.5	6.8
6	0.3	9.3	21.9	10.2
8			11.0	7.0

Mean hyperglicemic value change: 5.8.

In both the sole glucagon group and the hyaluronic acid-added glucagon group, the hyperglicenic aacid value was inceased by about 50 mg/ml in 0.25 hour after the administration. Subsequently, in the sole glucagon group the hyperglicemic value was gradually reduced to recover the initial value in 6 hours after the administration. In contrast, in the hyaluronic acid-added glucagon group, the hyperglicemic value was reduced with the lapse of time but was about 25.22 and 11 mg/ml after 4.6 and 8 hours from the

administration, respectively.

Example 3

As predonisolone for injection was used predonisolone acetate (Takeda). As hyaluronic acid-added predonisolone for injection was used a 4 % hyaluronic acid solution obtained by adding hyaluronic acid powder (molecular weight: 1,700,000) to the presonisolone for injection noted above.

As the experiment animal were used six normal male rabits (New Zealand while rabits, weight: 2.8 to 31. kg) in two groups each of three, i.e., sole predonisolone group and hyaluronic acid-added predonisolone group. Fest was made for 24 hours before the administration. In both groups, the administration was made in an amount of 15 mg as predonisolne by hypodermic administration on the back. Blood was extracted from auricular vein in an amount of about 12.5 mg before the administration and 0, 5, 1, 2 and 4 hours after the administration. The extracted blood was subjected to centrifugal separation at 300 rpm for 10 minutes. Plasma thus obtained was frozen to a constant amount and then preserved. Measurement of predonisolone in the obtained plasma was carried out by a HPLC process on the basis of a method shown in Hiroharu Kubo et al, "Analytic Chemistry", 30, 658 (1981).

The result is shown in Table 3.

Table 3

Time (h)	Sole predonisolone group predonisolone-in-blood concentration (mg/ml)		HA-added (4 %) predonisolone group predonisolone-in-blood concentration (mg/ml)	
0.5	175.3	72.0	9.4	10.5
1	205.8	155.2	28.2	19.2
2	150.5	61.9	376.8	262.2
4	49.6	60.7	198.6	127.6

Mean concentration: 5.E.

Table 4 shows pharmacokinetic parameters obtained from the transition of the concentration of the hypodermically administrated predonisolone in blood.

Table 4

Parameter	Sole Predonisolone group		HA-added (4 %) predonisolone group	
I... (h)	1.8	1.3	1.7	0.4
C... (ng/ml)	288.6	110.3	378.1	250.5
AUC.... (ng·h/ml)	517.2	190.7	789.6	490.2

Mean nvalue: 5.E.

I...: Time until reaching of the maximum concentration

C...: Maximum concentration

AUC...: Area under concentration-time curve until four hours after administration

Considering changes in the concentration of predonisolone in blood up to two hours from the administration in the sole predonisolone group and hyaluronic acid-added predonisolone group, with the sole predonisolone group the concentration is gradually increased compared that obtained with the sole predonisolone group. This means a delay of absorption of predonisolone based on the action of hyaluronic acid to cause gradual emission of predonisolone. Further, the concentration after 4 hours from the aministration was about 50 ad 200 mg/ml with the respective sole predonisolone and hyaluronic acid-added prednislone groups, a higher value being obtained with the hyaluronic acid-added predonisolone group. Further, with the hyaluronic acid-added predonisolone group the C... and AUC... were about 1.3 and 1.5 times, respectively, the values obtained with the sole prednisolone group. Since the above results are obtained with the same amount of administration for the two groups, it is considered that biological utilization

capacity of the hopydermically administrated predonisolone can be improved by adding hyaluronic acid.